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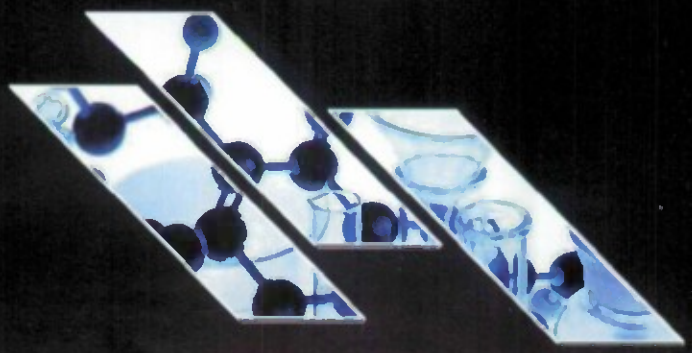
# DECONTAMINATION EFFICACY OF THREE COMMERCIAL OFF-THE-SHELF SPORICIDAL AGENTS ON MEDIUM-SIZED PANELS CONTAMINATED WITH SURROGATES OF *BACILLUS ANTHRACIS*



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A significant gap in technology preparedness exists in the U.S. Federal response to wide-area contamination resulting from the release of biological agents such as *Bacillus anthracis* spores. In 2001, release of just a few letters containing anthrax spores resulted in the contamination of several building interiors, including the U.S. Postal and Distribution Centers (Brentwood, Washington, DC; Trenton and Jersey City, NJ) and American Media Inc. (Boca Raton, FL). Despite heavy contamination levels of several building interiors, remediation of building interiors was achieved successfully by fumigation with chlorine dioxide (CD) or vapor hydrogen peroxide (VHP). A wide-area release and contamination of building exteriors and the outdoors would likely exhaust the national remediation capacity. Cleanup could take years and lead to incalculable financial drain because of a delay in effective response. Additionally, agencies responsible for the mitigation of contaminated sites are exploring alternative methods for decontamination including combinations for the disposal of contaminated items, source reduction by vacuuming, mechanical scrubbing, and pH-adjusted bleach pressure wash. If proven effective, a pressure wash-based removal of anthrax spores from building surfaces with readily available equipment will significantly increase the readiness of federal agencies to meet the daunting challenge of restoration and cleanup efforts following a wide-area biological release.

**15. SUBJECT TERMS**

| | | | |
|---|---|---|---|
| *Bacillus anthracis* spores | Sporicidal efficacy | Stainless steel | CASCAD |
| *Bacillus atrophaeus* spores | pH-amended bleach | Brick | Peridox |
| *Bacillus subtilis* spores | PT lumber | | |

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# PREFACE

The work described in this report was started in June 2009 and completed in September 2010.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.


## Acknowledgments

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# DECONTAMINATION EFFICACY OF THREE COMMERCIAL OFF-THE-SHELF SPORICIDAL AGENTS ON MEDIUM-SIZED PANELS CONTAMINATED WITH SURROGATES OF BACILLUS ANTHRACIS

## 1. INTRODUCTION

A number of standardized test methods (ASTM 2197-02; ASTM 2414-05; AOAC *Official Method* 2008-05) are available for determining sporicidal disinfectant and gaseous fumigant efficacy under pristine laboratory conditions. However, these methods are not suited for conducting efficacy studies of biologically contaminated wide-area urban environments such as building structures, which are composed of a vast array of porous and nonporous materials. Neither these methodologies nor commercial off-the-shelf (COTS) sporicidal agents can deal with the type and scale of mitigation and remediation needed. Under the auspices of the Interagency Biological Restoration Demonstration (IBRD) program, the present project was initiated to develop "new" methodologies and generate quantitative efficacy data to address the gap in wide-area decontamination.

The specific aspects of method development included

- Mid-sized panel assembly
- Spore inoculation and sampling
- Decon application
- Sample concentration and spore enumeration
- Waste disposal

Mid-sized panels were assembled and inoculated with *Bacillus atrophaeus* subspecies *globigii* (Bg) spores as liquid suspension. Polyurethane wipes and vacuum socks were used for spore recovery from the panels. Depending on the surface composition and the decontamination technology tested, viable spore recovery from the panels varied after the decontamination trials.

Some of the panels were maintained as controls and the following safeguards were set in place:

- Sampling before decontamination application to quantify the efficacy of the sampling technologies used in this study

- Spraying with water to enumerate the spores physically removed from the surface of the panels by the mechanical interactions of the spores, liquid application, and surface material

Restoration of buildings for re-occupancy requires a high degree of public trust in federal agencies authorized to declare that decontaminated areas pose minimal or no risk for infection. In an attempt to achieve higher levels of decontamination, a

second decontaminant was applied to each panel. With one exception, the number of viable spores recovered decreased to just a few spores per panel and, in some cases, to below the detectable level of the sampling technologies used in this study. The method and efficacy data from experiments using three decontamination technologies on mid-sized panels are summarized in this report.

## 2. METHODS AND MATERIALS

### 2.1 Bacterial Strains

Ten grams of *Bacillus atrophaeus* subspecies *globigii* Dugway 1088 (BG) spores were washed, pelleted, and resuspended six times in sterile distilled water. After the final wash, the spores were suspended in 50 mL water (spore concentration measured $1.5 \times 10^9$ colony forming units [CFU]/mL) and stored at 4 °C until used. The spore stocks were periodically checked by performing a staining procedure. Working stocks of approximately $1.65 \times 10^9$ CFU/mL were achieved by diluting the working stock with appropriate volumes of water.

### 2.2 Panel Construction

The mid-sized panels (33 each) were constructed from the following materials:

- Brick veneer
- Stainless steel
- Pressure-treated (PT) wood lumber

All the panels were made with 7/16 in. thick 4 $ft^2$ oriented strand board (OSB) as a backing material (Home Depot, Bel Air, MD [Cat. No. 386-081]). The stainless steel (Durrett Sheppart Steel, Baltimore, MD) panels were composed of eight individual 1 x 2 ft (T-304 No. 2B finish 20 gauge) stainless steel sheets glued to the OSB backing board with construction adhesive to form a single 4 $ft^2$ panel. PT lumber (Home Depot [Cat. No. 155-400]) panels were constructed by assembling 8 boards measuring 48 in. (length), 5½ in. (width), and ¾ in. (thick) and one board measuring 48 in. (length), 4 in. (width), and ¾ in. (thick) to achieve a PT lumber panel measuring 48 in. (length) and 48 in.(height). The PT lumber was secured to the OSB with a single 1¼ in. exterior screw (Home Depot [Cat. No. 131-537]) at each end of the board. The brick panels were constructed by securing a metal grid (Brickit.com, Bohemia, NY [Cat. No. MGMOD48X8]) to OSB panels with construction adhesive and ½ in. exterior screws. A ½ in. thick brick veneer (Brickit.com [Cat. No. TSMODKINGW]) was then secured to the metal grid using construction adhesive.

## 2.3 Panel Seeding

The panels were divided into three testing categories:

- Control panels treated with no disinfectants
- Panels treated with a decontamination technology
- Panels wetted with distilled water

For the testing, each panel was seeded with 1280 individual 10 µL drops of evenly distributed BG spores to achieve a total spore load of approximately $2.1 \times 10^9$. Once inoculated, the panels were set aside to allow the spore suspension to dry for 24 h. Only the panels to be tested the following day were inoculated with spores at one given time.

## 2.4 Application of Decontamination Technologies

Inoculated panels were attached vertically to an in-house panel holder with two clamps in the upper right and left corners.* The runoff from the application of the decontaminant was collected at the bottom of each panel (Figure 1).


Figure 1. Panel holder for decontamination application.

* The in-house panel holder was designed by Dr. V.K. Rastogi and fabricated by the Advanced Design & Manufacturing Team (Aberdeen Proving Ground, MD).

The control panels were treated with water. For each test, a contaminated panel was treated with one of the following liquid solutions:

- Distilled water
- Peridox (Clean Earth Technologies, Earth City, MO [Cat. No. Per-1])
- 1:10 pH-amended Ultra Clorox Germicidal bleach (Pittsburgh, PA [Cat. No. NC9842935])
- CASCAD (Allen Vanguard Technologies, Ottawa, ON, Canada [Cat. Nos. GP2100-730, GCE2000-950, and GPX-4000])

Each panel and decontamination technology combination consisted of three experimental repeats using 10 panels for each set, totaling 30 panels for each decontamination technology tested. Both controls (water and no liquid application) were performed with each experimental repeat run, consisting of a single panel per run for a total of three panels each.

Peridox and CASCAD were diluted according to the manufacturer's directions. Each disinfectant liquid was applied with a low-pressure 4 gal backpack sprayer (Agri Supply Co., Garner, NC [Cat. No. 59540]) to the appropriate panel from approximately 18 in. from the panel. Each panel was visually monitored to ensure that it remained wet with the decontamination solution for a contact time of 30 min. The panels were then set aside and allowed to dry for $\geq$2 h prior to sampling.

After 3–4 h, the panels were subsequently treated with a reapplication of the respective decontamination technology. The only exception was with CASCAD. Half of the CASCAD-treated panels received a reapplication of CASCAD solution while the other half were rewetted with distilled water. Post-reapplication sampling of the panels was performed identically as described for the initial application. After sampling was performed, the panels were set aside for 2–4 weeks.

## 2.5 Sampling Methodology

Hard surface steel panels were sampled using wipes, and the two porous materials were sampled using a vacuum sock. The panels were separated into three groups (Figure 2):

- Control panels treated with no disinfectants
- Panels treated with a decontaminant
- Panels wetted with distilled water

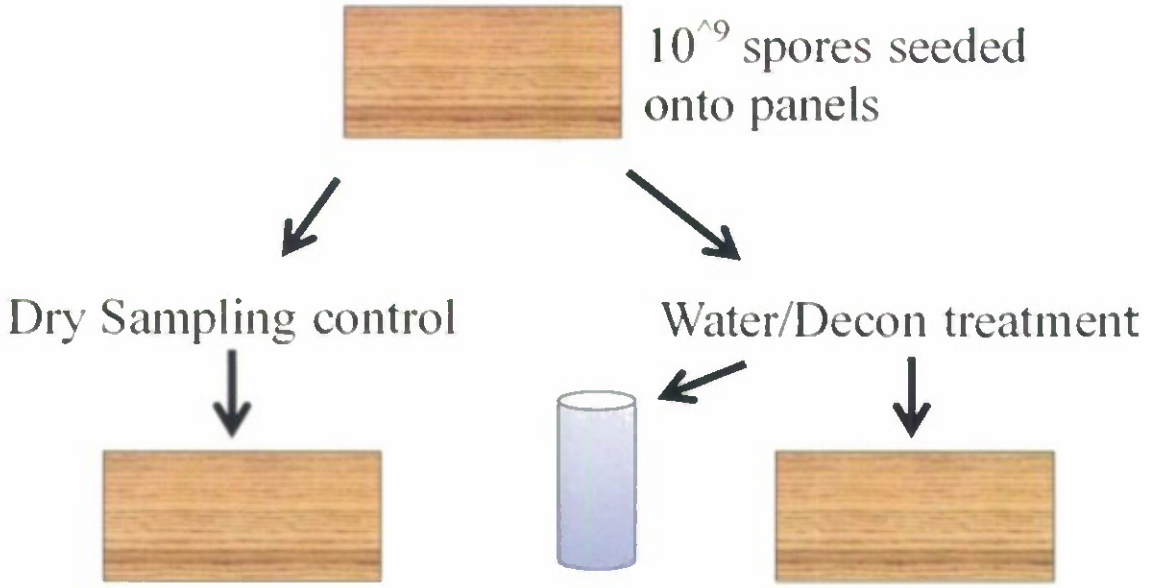


Figure 2. Sampling flow chart.

Each stainless steel panel was divided into 1 $ft^2$ sections. Each section was sampled using 1/8 of a polyurethane wipe (VWR International, LLC, Bridgeport, NJ [SterileWipe* LP Wiper, ITW Texwipe*] [Cat. No. TWTX3211]), which added up to a total of four full wipes per panel. Each wipe section was folded into quarters, providing four wiping surfaces. Each surface was used on the same 1 $ft^2$ section of stainless steel panel. After swiping, the wipes were placed into individual 50 mL conical tubes, each containing 10 mL 0.1% phosphate buffer saline (PBS)-Triton-X-100.* The 16 partial wipes were processed as individual samples and the data were pooled after analysis.

The two porous surface materials, PT lumber and brick, were sampled with a vacuum sock technology (Midwest Filtration Co., Cincinnati, OH [HEPA filter sock collection kit, and Omega Hepa Vacuum] [Cat Nos. FAB-20-01-001A and 950-A1-00-120]). Each panel was sampled with a single vacuum sock. The nozzle of the collection tube was held approximately ½ in. above the surface. The nozzle was slowly moved back and forth across the top surface using left-to-right horizontal strokes to collect spores. This procedure was repeated two more times using top-to-bottom vertical and left-to-right horizontal strokes. The nozzle was removed from the vacuum hose before the vacuum sock was removed from the filtration nozzle. The vacuum sock filter was placed into the appropriate 50 mL conical tube containing 35 mL PBS with 0.01% Tween (Sigma Chemicals, Perth, WA) and pushed down so it was submerged in the fluid.

* PBS, a common reagent, was made in-house by the Advanced Design & Manufacturing Team (Aberdeen Proving Ground, MD).

Runoff samples were collected from the collection tray located under the panel stand. The control panels were kept wet for 30 min by repeated sprays before the runoff samples were collected and measured. The aliquots were serially-diluted and plated as previously described. An aliquot of 25 mL runoff sample was removed from each of the panels treated with a decontaminant and filtered through a 0.2 μm syringe filter. Each filter was rinsed twice by passing 25 mL of sterile distilled water through it. The filters were then placed in conical tubes with extraction buffer, and the spores collected by the filters were recovered as previously described.

### 2.6 Sampling Analysis

Samples from each wipe and vacuum filter were serially-diluted and plated in triplicate, and the mean of each triplicate plate was recorded. The mean CFU counts for each data set were calculated by averaging the respective runoff and surface material sampled spores. CFU calculations for wipes used with each stainless steel panel were combined after plating for total panel recovery calculations. Percent recovery (%RE) was calculated by dividing the mean recoverable CFUs from the sampling material by the total number of spores inoculated onto the panels.

## 3. RESULTS

### 3.1 Sampling Recovery Efficiency

Recovery efficiencies of sampling technologies on the panels were estimated by calculating the amount of viable spores recovered from the untreated control panels that had not undergone any type of liquid decontamination treatment. Over 9.2 logs of spores were recovered from the stainless steel panels, which represents approximately 76% of the spores inoculated onto the panels (Table 1). Recovery efficiencies from the brick and lumber panels were significantly lower, approximately 7.3 and 7.5 logs, respectively, which accounted for <1% of the spores inoculated on the panel.

Table 1. Percent Spore Recovery from Untreated Panels

| | **Recovery (%)** | **Spores Recovered (Logs)** |
|---|---|---|
| Steel | 76 | 9.2 |
| Brick | 1 | 7.3 |
| Lumber | 1 | 7.5 |

## 3.2 Control Spores Collected in Runoff

The inoculated panels were sprayed with water to determine what effect mechanical interactions played in spore removal from each panel type. Approximately 8.7 logs were recovered from the water runoff from the stainless steel panels, which represented 24% spores (Table 2). From the, brick and lumber panels, approximately 8.2 logs or 8% spores and 8.6 logs or 16% spores, respectively, were recovered from the water runoff (Table 2).

Table 2. Spores Recovered in Runoff

| | Recovery (%) | Spores Recovered (Logs) |
|---|---|---|
| Steel | 24 | 8.7 |
| Brick | 8 | 8.2 |
| Lumber | 16 | 8.6 |

## 3.3 Efficacy and Efficiencies of Decontamination Technologies

All the panel types were treated with two applications of decontamination solution. The first application of decontamination solution on stainless steel panels resulted in significant log reduction (LR) in viable spore numbers (e.g., 4.8, 4.7, and 9.1 logs) when treated with Ultra Clorox Germicidal bleach, Peridox, and CASCAD (Table 3). After the second application, the LR values significantly increased to 8.6 logs with bleach, modestly increased to 6.6 logs with Peridox, and remained the same with CASCAD (Table 4).

Table 3. Spore Recovery after Initial Decontamination Application

| | Water (Logs) | Bleach (Logs) | Peridox (Logs) | CASCAD (Logs) |
|---|---|---|---|---|
| Steel | 0.1 | 4.8 | 4.7 | 9.1 |
| Brick | 0.7 | 8.6 | 9.3 | 9.2 |
| Lumber | 1.9 | 4.9 | 8.0 | 9.0 |

Table 4. Spore Recovery after Reapplication

| | Bleach (Logs) | Peridox (Logs) | CASCAD (Logs) | CASCAD/Water (Logs) |
|---|---|---|---|---|
| Steel | 8.6 | 6.6 | 8.9 | 8.4 |
| Brick | 9.1 | 9.0 | 9.2 | 9.1 |
| Lumber | 8.7 | 8.9 | 9.2 | 9.1 |

The sporicidal efficacy on lumber was similar to that on stainless steel. Bleach was less effective after the initial application with an LR value of 4.9. Extremely few CFUs were recovered from the initial application of CASCAD with an LR value of 9.0. However, Peridox was the most effective in the mitigation of contaminated PT lumber. After the second application, all three decontaminants performed similarly with LR values of 8.7, 8.9, and 9.2, for Ultra Clorox Germicidal bleach, Peridox, and CASCAD, respectively.

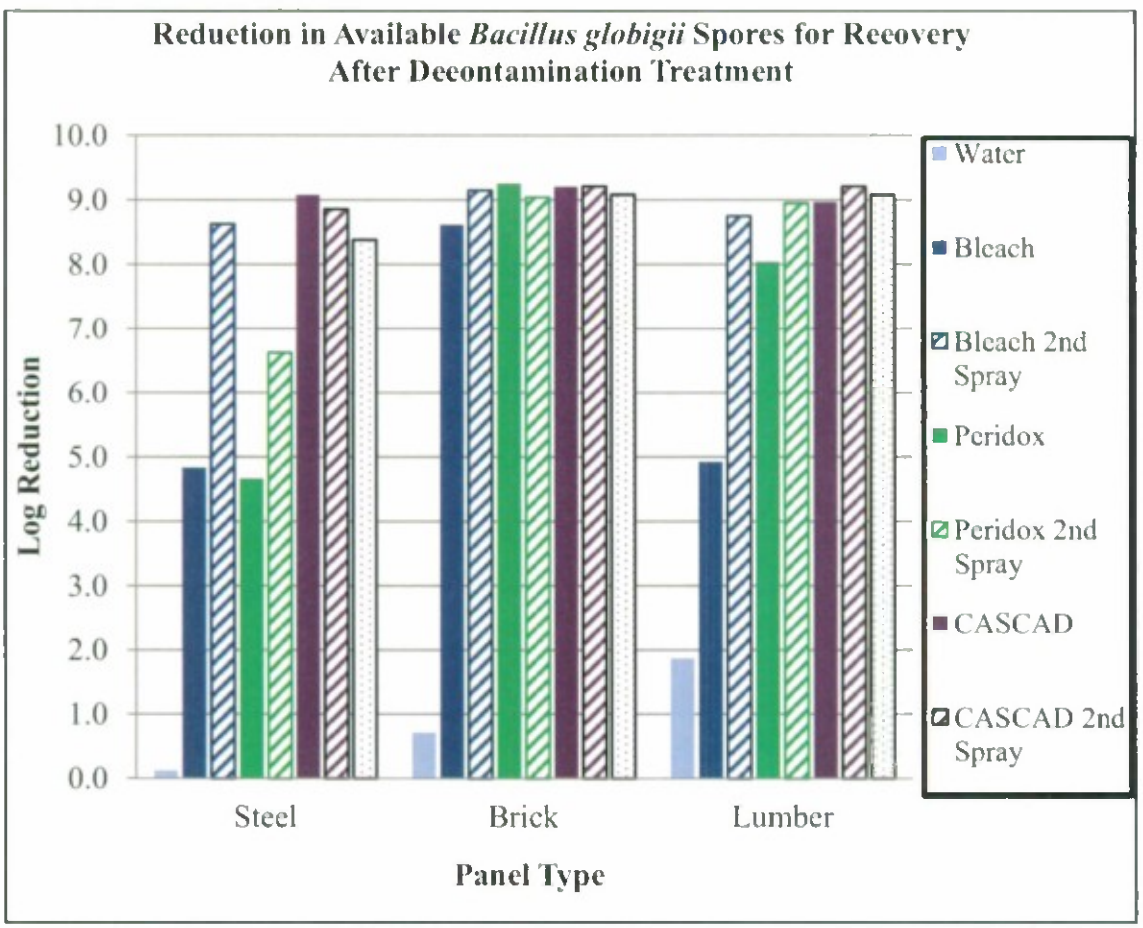


Figure 3. Log reduction in viable spore numbers after different decontamination treatments.

## 4. DISCUSSION

The sporicidal efficacy testing data summarized in this report compared three COTS technologies: Ultra Clorox Germicidal bleach (a standard benchmark), Peridox (an acid and peroxide decontaminant), and CASCAD (a chlorine and acid-based foam). The manufacturer's recommendation for a ≥3 h contact time was considered unreasonable and unrealistic because vertical surfaces cannot be kept wet for this length of time. For this study, a more reasonable time for decontamination was thought to be 30 min. A low pressure backpack sprayer was used to apply the decontaminant in an effort to better control the liquids and ensure that spores could be collected in the runoff or remain on the panel surface. Approximately 2 gal of decontaminant were applied to ensure a contact time of 30 min for bleach and Peridox. Respraying was performed every 2–5 min depending on the temperature and humidity on the day of testing. With CASCAD, approximately 1.5 gal per panel of the decontaminant was used, and it required only a single reapplication because of its foaming property.

After the first 30 min application of decontaminant was evaluated, CASCAD was found to greatly outperform the other two decontaminants on stainless steel and significantly outperformed bleach on lumber. This came as no surprise because previous decontamination attempts using bleach on pinewood were reported as ineffective (Tomasino *et al.*, 2010). However, all three decontamination technologies had relatively similar effect on brick. Although brick and PT lumber are porous, the effectiveness of bleach to decontaminate these materials greatly differed. This was not true for Peridox and CASCAD, which suggested that porosity, alone, is not responsible for decontamination efficacy. The chemical constituents within PT lumber neutralize bleach. CASCAD may have outperformed the other two decontamination technologies because it foams, sticks better, and has a 10x concentration of chlorine.

One single potential source of error in this study was the use of the vacuum sock technology for spore recovery from the porous materials. A 2 log reduction in the number of spores recovered from untreated (no decontaminants used) brick and lumbar panels showed significant spore loss. Areas with $10^9$ spores per 16 $ft^2$ would be considered heavily contaminated; however, vacuum sock technology cannot be used to document this characterization. Clearly, the negative results derived from this technology cannot reassure U.S. Federal agencies and the public about post-decontamination sampling based cleanups. A study by Brown *et al.* (2007) evaluated the vacuum filter sock technology and identified several characteristics, including pore diameters over 50 μm in the filter, which contribute to the inefficiency of this particular sampling device.

In addition to a number of factors affecting sampling efficiencies, inherent characteristics of the surface material, including porosities and effects of spore surface on adhesion forces to a given surface type, are completely unknown (Edmonds, 2009). A large gap exists with respect to our understanding of how porosities of surface materials can protect spores from coming into contact with decontaminants.

Additionally, if a biological agent is applied to a wet surface or to a porous material as a wet aerosol, or if it comes into contact with rain prior to being decontaminated, the amount of spore removal resulting from water transport through the matrix of a porous surface is unknown. Data are also lacking with respect to the distance an agent travels away from the surface, thus avoiding decontamination. Environmental conditions can contribute to the persistence of the agent as either an aerosol or cutaneous threat well after decontamination efforts have ceased. An improvement in vacuum sampling devices and a basic fundamental understanding of adhesive forces and physical interaction between agent and surface material are absolutely necessary to improve efficacy of future decontamination studies, especially in the context of wide-area decontamination assessments following a biological release.

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